tus branchs, approximately 1.5 m in length and 5.0 cm in diameter, were provided to each pen, and exchanged for new ones after 15 days. The hay that remained outside the burrows was swept out of the pens every morning. Every ten days, the whole pen floor was washed, even inside the burrows after the hay had been removed, and the water tanks were brushed. All maintenance of the animals was performed by the same two staff persons, who had already been doing this work for at least one year before the study started.

Observations were registered by continuous recording (MARTIN and BATESON 1986), with all registry done in a descriptive manner by the same observer. Observations began in February 1998, and were conducted from 7.30 h a.m. to 3.00 h p.m. (daylight) for 40 consecutive days, for a total of 148 hours. Nocturnal observations were made from March to July of the same year, from 5.30 h p.m. to 10.00 h p.m., over scattered days for a total of 31 hours. The night schedule was selected, based on a previous study for 72 hours of continuous observation of the activity rhythm. These pacas showed an activity peak from 5.30 h p.m. to 10.00 h p.m. In addition, the animals were observed for two more days, between 6.00 h p. m. to 12.00 h a. m. and 12.00 h a. m. to 6.00 h a.m. In order to acclimate the animals to artificial light, two nights before each nocturnal observation two lamps (with 40 watt each, positioned 5 m equidistant) illuminated the four pens. Observations inside the burrows were possible since one corner of the wood cover was lifted 30 cm with a wire.

Although defecation occurred mainly at night, caecotrophy was detected only once during the nocturnal observations, and this caecotrophy was of already defecated faeces. Ingestion of faeces directly from the anus was, in contrast, only observed during daytime, always occurring inside the burrows. The paca can rest in the burrow using three different positions: with the belly upward and the four limbs flexed near the body; with the bodyside and cheek on the floor and the four limbs stretched perpendicular to the body; and, with the sternum on the floor and the limbs close to the body (i.e.: the sternal position). Caecotrophy occurred when the animals were resting in the sternal position, by raising the chest off the ground, then putting the snout between the hind legs and repeatedly licking the anus; and finally lifting the head and chewing for about ten seconds, swallowing soon after. This cycle was repeated up to ten times. All adults and immature pacas over two months old showed this behaviour daily, throughout the diurnal observation period, however, one adult female performed caecotrophy during nocturnal observations.

Consumption of faeces by captive pacas has previously been reported (Матамокоs 1982), but no mention was made of caecotrophy of differentiated faeces.

Since pacas have large intestines with a functional caecum (BENTTI 1981) and since they are phylogenetically related to hystricognaths who perform caecotrophy, the consumption of differentiated faces should be interpreted as related to the feeding habits of pacas and not as an abnormal behaviour resulting from captivity (GRIER 1984). Although studies concerning the natural feeding habits of this species are lacking, pacas have been considered to be frugivores. The occurrence of caecotrophy and their digestive tract anatomy suggests that pacas may be more herbivorous than expected, often browsing on leaves, and not only when fruits are scarce.

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### References

- AYRES, J. M.; LIMA, D. M.; MARTINS, E. S.; BAR-REIROS, J. L. K. (1991): On the track of the road: Changes in subsistence hunting in a Brazilian amazonian village. In: Neotropical Wildlife Use and Conservation. Ed. By J. G. ROBINSON and K. H. REDFORD. Chicago: Univ. Press. Pp. 82–92.
- BENTTI, S. B. (1981): Las lapas, roedores de América tropical. Natura 70/71, 40–44.
- BERGALLO, H. G.; DUARTE DA ROCHA, C. F.; AL-VES, M. A. S.; VAN SHYS, M. (2000): A fauna ameaçada de extinção do Estado do Rio de Janeiro. Rio de Janeiro. Editora da Univ.
- BJÖRNHAG, G.; SJÖBLOM, L. (1977): Demonstration of caecotrophy in some rodents. Swedish J. Agric. Res 7, 105–113.
- BORGES, P. A.; DOMINGUEZ-BELLO, M. G.; HER-RERA, E. A. (1996): Digestive physiology of wild capybara. J. Comp. Physiol. 166, 55–60.
- CRANFORD, J. A.; JOHNSON, E. O. (1989): Effects of coprophagy and diet quality on two microtine rodents (*Microtus pennsylvanicus* and *Microtus pinetorum*). J. Mammalogy **70**, 494–502.
- EMMONS, L. H. (1990): Neotropical Rainforest Mammals – a field guide. Chicago, London: Univ. of Chicago Press.
- Grier, J. W. (1984): Biology of Animal Behavior. Times Mirror/Mosby College Publ, Missouri: D. Bowen.
- HARCOURT, A. H.; STEWART, K. J. (1978): Coprophagy by wild mountain gorillas. E. Af. Wildl. J. 16, 223–225.
- MAROUNEK, M.; VOVK, S. J.; SKRIVANOVA, V. (1995): Distribution of activity of hydrolytic enzymes in the digestive tract of rabbits. British J. Nutr. **73**, 463–469.
- MARTIN, P.; BATESON, P. (1986): Measuring Behaviour – an introductory guide. Cambridge: Cambridge Univ. Press.
- MATAMOROS, Y. (1982): Notas sobre la biologia del tepezcuinte, *Cuniculus paca*, Brisson (Rodentia: Dasyproctidae) en cautiverio. Brenezia **19/20**, 1–82.
- Mondolfi, E. (1972): La lapa o paca. Def. Nat. 2, 4–16.

- MOROT, M. CH. (1882): Des pelotes stomachales des léporidés. Mém Soc. Centr. Méd. Vét. 12, 139–239.
- OSAWA, R.; BLANSHARD, W. H.; OCALLAGHAN, P. G. (1993): Microbiological studies of the intestinal microflora of the koala, *Phascolarctos cinereus*. Pap, a special maternal faeces consumed by juvenile koalas. Austr. J. Zool. **41**. 611–620.
- PICKARD, D. W.; STEVENS, C. E. (1972): Digesta flow through the rabbit large intestine. Am. J. Physiol. 222, 1161–1166.
- SMYTHE, N.; GLANZ, W. E.; LEIGH JR., E. G. (1983): Population regulation in some terrestrial frugivores. In: The Ecology of a Tropical Forest: Seasonal Rhythms and Long-Term Changes. Ed. By E. G. LEIGH JR., A. A. RAND, and D. M. WINDSOR. Washington D. C.: Smithsonian Inst. Press. Pp. 227–238.
- SOAVE, O.; BRAND, C. D. (1991): Coprophagy in animals a review. Cornell Vet. 81, 357–364.
- STILLINGS, B. R.; HACKLER, L. R. (1966): Effect of coprophagy on protein utilization in the rat. J. Nutr. 90, 19–24.
- TAKAHASHI, T.; SAKAGUSHI, E. (1998): Behaviors and nutritional importance of coprophagy in captive adult and young nutrias (*Myocastor coypus*). J. Comp. Physiol. **168**, 281–288.
- VICKERS, W. T. (1991): Hunting yields and game composition over ten years in a Amazon indian territory. In: Neotropical Wildlife Use and Conservation. Ed. By J. G. ROBINSON and K. H. REDFORD. Chicago: Univ. Press. Pp. 53– 81.
- Woods, C. A. (1984): Hystricognath rodents. In: Orders and Families of Recent Mammals of the World. Ed. By S. ANDERSON and J. K. JONES Jr. Canada: John Wiley. Pp. 389–445.

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# Short communication

# Protein polymorphism in two species of *Ctenomys* (Rodentia, Ctenomyidae) from Córdoba province, Argentina

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Key words: Ctenomys bergi, Ctenomys rosendopascuali, allozymic polymorphism, speciation

Fossorial rodents of the genus *Ctenomys* are widespread in southern South America, from 17° to 54° S (CABRERA 1961; REIG et al. 1990). The genus comprises 60 recognized species originated by an explosive speciation process, promoted mainly by chromosomal rearrangements (BIDAU et al. 1996). At present, systematic relationships among species of *Ctenomys* are poorly known and/ or controversial.

THOMAS (1902) cited the species *C. bergi* for the NW of Córdoba province, Argentina, being Cruz del Eje the type locality. On the basis of geographic criteria, all the populations from the north of that province were included in that species (BIDAU et al. 1996).

Chromosomal studies revealed that individuals from the NE of Córdoba have a diploid number 2 n = 52 (FN = 66) but those proceeding from the NW (Salinas Grandes) presented a karyotype of 2 n = 48 (FN = 90) (REIG et al. 1990). This last form was assigned to *C. bergi* and the former was described as a new species and denominated *C. rosendopascuali* (CONTRERAS 1995).

Several authors have emphasized the importance of the application of biochemical and molecular methods in order to confirm and clarify the taxonomic status of different karyotypic forms of *Ctenomys* (BIDAU et al. 1996; MASCHERETTI et al. 2000). The aim of this study is to analyze the allozymic polymorphism in two populations of *Ctenomys* from the north of Córdoba, Argentina assigned to *C. bergi* and *C. rosendopascuali*, in order to determine their level of differentiation at structural loci.

Fourteen specimens of *C. bergi* from Las Toscas ( $30^{\circ}11'$  S,  $64^{\circ}54'$  W, near an extense salt mine called Salinas Grandes) and 16 individuals of *C. rosendopascualis* obtained in the proximity of the mouth of Xanaes river (Mar Chiquita saline lagoon,  $30^{\circ}55'$  S  $62^{\circ}44'$  W) were used in this study.

Animals were killed by ether anesthesia, liver and kidneys removed immediately and preserved at -30 °C until used. Homogenates, vertical starch gel electrophoresis and staining procedures were carried out as described by GARDENAL et al. (1980) and GARDENAL and BLANCO (1985). The following enzymes were analyzed (loci scored and E. C. numbers in parenthesis): liver and kidney acid phosphatase (Acp<sub>L</sub>-1, Acp<sub>L</sub>-2, Acp<sub>K</sub>-3, Acp<sub>K</sub>-4; 3.1.3.2), aspartate aminotransferase (Aat-1, Aat-2; 2.6.1.1), liver soluble esterases (Es-1<sub>L</sub> to Es-6<sub>L</sub>; 3.1.1.1), catalase (Cat; 1.11.1.6), phosphoglucomutase (Pgm-1, Pgm-2; 2.7.5.1), leucine aminopeti-

dase (Lap-1, Lap-2; 3.4.11.1), malic enzyme (Me; 1.1.1.40), lactate dehydrogenase (Ldh; 1.1.1.27), alcohol dehydrogenase (Adh; 1.1.1.1), glycerophosphate dehydrogenase (Gpdh; 1.1.1.8), malate dehydrogenase (Mdh-1,Mdh-2; 1.1.1.37), isocitrate dehydrogenase (Idh-1, Idh-2; 1.1.1.42), 6-phosphogluconate dehydrogenase (6-Pgdh; 1.1.1.43) and glucose-6-phosphate dehydrogenase

(G-6-pdh; 1.1.1.49). The allele coding for the band migrating fastest to the anode was assigned the number 100; that controlling the fastest cathodic band, -100. The other alleles were numbered according to their relative mobility from the origin. Bands with the same mobility were considered homologous.

Proportion of polymorphic loci (95% and 99% criteria), mean observed and expected heterozygosities, Rogers' genetic distance (1972) and Nei's identity (1975) among populations were calculated using the program Biosys-1 (SwoFFORD and SELANDER 1989).

Sixteen out of 27 loci analyzed were polymorphic at least in one population. Table 1 shows allele frequencies, proportion of polymorphic loci (P), and observed and expected mean heterozygosity per locus ( $H_o$  and  $H_e$ ) for the two populations analyzed. Locus G-6-pdh was the only one presenting a different allele fixed in each population.

Although crossing tests were not performed, the genetic control of the electrophoretic patterns observed was postulated on the basis of similar polymorphisms described for other rodent species where the Mendelian transmission of variants has been demonstrated (GARDENAL and BLANco 1985; GARDENAL et al. 1980; GARCÍA and GARDENAL 1989). In all cases, the observed genotypic frequencies did not differ significantly from the expected ones according to the Hardy-Weinberg equilibrium.

Rogers' genetic distance and similarity between the two species was 0.094 and Nei's distance and identity were 0.059 and 0.942, respectively.

Levels of polymorphism revealed in this study for *C. bergi* and *C. rosendopascuali* are particularly high when compared with those reported for other subterranean mammals with low vagility and sociallystructured mating system (Nevo et al. 1990). Values of heterozygosity obtained in this study are higher than the mean referred for fossorial rodents (H = 0.0311) and for

**Table 1.** Allele frequencies, proportion of polymorphic loci (95% and 99% criteria) and observed and expected heterozygocity in *Ctenomys bergi* and *Ctenomys rosendopascuali* from Córdoba province (Argentina).

| Locus               | Allele | C. bergi   | C. rosendopascuali |
|---------------------|--------|------------|--------------------|
| Lap-2               | 100    | 1.000      | 0.929              |
|                     | 88     | 0.000      | 0.071              |
| Acp <sub>K</sub> -1 | 100    | 0.067      | 0.036              |
|                     | 90     | 0.900      | 0.857              |
|                     | 81     | 0.033      | 0.107              |
| Adh                 | -100   | 0.867      | 0.864              |
|                     | -50    | 0.133      | 0.136              |
| Gpdh                | 100    | 1.000      | 0.923              |
|                     | 60     | 0.000      | 0.077              |
| Acp <sub>L</sub> -3 | 100    | 0.094      | 0.038              |
|                     | 78     | 0.906      | 0.962              |
| Acp <sub>L</sub> -4 | 100    | 0.031      | 0.000              |
|                     | 71     | 0.969      | 1.000              |
| Aat-1               | 100    | 0.000      | 0.0154             |
|                     | 72     | 0.969      | 0.0846             |
|                     | 20     | 0.031      | 0.000              |
| Es-1                | 100    | 0.844      | 0.855              |
|                     | 93     | 0.156      | 0.115              |
| Es-2                | 100    | 0.563      | 0.731              |
|                     | 94     | 0.438      | 0.269              |
| Es-3                | 100    | 0.906      | 0.885              |
|                     | 88     | 0.094      | 0.115              |
| Es-4                | 100    | 0.000      | 0.077              |
|                     | 89     | 0.656      | 0.615              |
|                     | 85     | 0.344      | 0.308              |
| Es-5                | 100    | 0.000      | 0.038              |
|                     | 89     | 1.000      | 0.962              |
| Es-6                | 100    | 0.813      | 0.269              |
|                     | 77     | 0.188      | 0.731              |
| Pgm-2               | 100    | 1.000      | 0.846              |
|                     | 82     | 0.000      | 0.154              |
| Me                  | 100    | 0.063      | 0.000              |
|                     | 89     | 0.938      | 1.000              |
| G 6pdh              | 100    | 1.000      | 0.000              |
|                     | 87     | 0.000      | 1.000              |
| P (95%)             |        | 33.33      | 40.74              |
| P (99%)             |        | 40.75      | 48.15              |
| H <sub>0</sub> (%)  |        | 10.1       | 12.8               |
| 0, 1,               |        | (s. e. 3)  | (s. e. 3.4)        |
| H <sub>E</sub> (%)  |        | 9.3        | 11.7               |
| - ( )               |        | (s.e. 2.8) |                    |